EXHIBIT 1

CURRICULUM VITAE

MARY ESTELLE WHITE-SCHARF, PH.D.

Home Address:
19 Johnson Rd.
Winchester, MA 01890
(617) 729-4079

Business Address:
Bldg. 75, 3rd Avenue
Charlestown Navy Yard
Charlestown, MA 02129
(617) 241-5200 x-209
(617) 241-8780 FAX

EDUCATION

Stanford University School of Medicine, Stanford, CA
Ph.D. Medical Microbiology, 1978
Title of dissertation: *Genetic and Immunologic Aspects of the Murine Response to a* Klebsiella Pneumoniae *Polysaccharide.*
Advisor: Dr. Leon T. Rosenberg

University of Texas Medical Branch, Galveston, TX
M.A. Physiology, 1974
Title of thesis: *Relationship of Scald Injury to Lymphocytic Distribution and Function*
Advisor: Dr. S. N. Kolmen

Southern Methodist University, Dallas, TX
B.S. Biology, 1972

EMPLOYMENT:

1995 - PRESENT — **Vice President of Research**
BioTransplant Incorporated, Charlestown, MA

1991 - 1995 — **Director, Monoclonal Antibodies Department**
BioTransplant Incorporated

1988 - 1992 — **Research Scientist**
Repligen Corporation, Cambridge, MA

1981 - 1988 — **Postdoctoral Fellow**
Massachusetts Institute of Technology, Cambridge, MA
Laboratory of Dr. Malcolm L. Gefter, 1985 - 1988
Laboratory of Dr. Thereza Imanishi-Kari, 1981 - 1985

1978 - 1981 — **Postdoctoral Fellow**
University of Cologne, Institute for Genetics, Cologne, Germany
Laboratory of Prof. Klaus Rajewsky

1978 — **Instructor of Microbiology**
California College of Podiatric Medicine, San Francisco, CA

HONORS AND AWARDS

Principal Investigator, STTR Grant *Use of Human Anti-CD34 mAb for Organ Transplantation*, 1996

Special Fellow, Leukemia Society of America, 1982-84

Fellow, Damon Runyon-Walter Winchell Cancer Fund, 1978-80

PUBLICATIONS

1. White, M. E. **Relationship of Scald Injury to Lymphocytic Distribution and Function.** 1974. Thesis, written in partial fulfillment of the requirements for M.A., University of Texas Medical Branch, Galveston, Texas.
2. White-Scharf, M. E. and L. T. Rosenberg. 1978. **Genetically Controlled IgM Hyporesponsiveness to a *K. Pneumoniae* Polysaccharide.** *Immunogenetics* 6:81.
3. White-Scharf, M. E., and L. T. Rosenberg. 1978. **Evidence that L-Rhamnose is the Antigenic Determinant of Hyporesponsiveness of BALB/c Mice to *Klebsiella Pheumoniae* Type 47.** *Infection and Immunity* 22:18.
4. White-Scharf, M. E. 1978. **Genetic and Immunologic Aspects of the Murine Response to a *Klebsiella Pneumoniae* Polysaccharide.** Dissertation, written in partial fulfillment of the requirements of Ph.D., Stanford University.
5. White-Scharf, M. E., and T. Imanishi-Kari. 1981. **Characterization of the Np^a Idiotype through the Analysis of Monoclonal BALB/c Anti-(4-hydroxy-3-nitrophenyl) Acetyl (NP).** *Eur. J. Immunol.* 11:897.
6. White-Scharf, M. E., and T. Imanishi-Kari. 1982. **Cross-Reactivity of the Np^a and Np^b Idiotypic Responses of BALB/c and C57BL/6 Mice to (4-hydroxy-3-nitrophenyl) Acetyl (NP).** *Eur. J. Immunol.* 12:935.
7. Loh, D. Y., A. M. Bothwell, M. E. White-Scharf, T. Imanishi-Kari, and D. Baltimore. 1983. **Molecular Basis of a Mouse Strain-Specific Anti-Hapten Response.** *Cell* 33:85.
8. Boersch-Supan, M., S. Agarwal, M. E. White-Scharf, and T. Imanishi-Kari. 1985. **Multiple V_H Gene Segments Encode NP-Idiotypic Antibodies.** *J. Exp. Med.* 161:1272.
9. White-Scharf, M. E., and T. Imanishi-Kari. 1986. **Genetic Basis for Altered Idiotype Expression in the Hyperimmune Response to (4-hydroxy-3-nitrophenyl) Acetyl Hapten.** *J. Immunol.* 137:887.
10. Souroujon, M., M. E. White-Scharf, J. Andre-Schwartz, M. L. Gefter, and R. S. Schwartz. 1988. **Preferential Autoantibody Reactivity of the Preimmune B Cell Repertoire in Normal Mice.** *J. Immunol.* 140:4173.
11. White-Scharf, M. E., M. Souroujon, J. Andre-Schwartz, R. S. Schwartz, and M. L. Gefter. 1988. **Specificity of the Germline-Encoded Preimmune B Cell Repertoire.** *UCLA Symp. Mol. Cell. Biol. (New Ser.)* 85:105.
12. Higgins, P. J., T. Paradis, B. J. Potts, M. E. White-Scharf, J. R. Rusche, and C. F. Scott. 1992. ***In Vitro* Inhibition of a Variety of Human Immunodeficiency Virus Isolates by a Broadly Reactive, V3-Directed Heteroconjugate Antibody.** *The Journal of Infectious Diseases* 166:198.
13. White-Scharf, M. E., B. J. Potts, L. M. Smith, K. A. Sokolowski, J. R. Rusche, and S. Silver. 1993. **Broadly Neutralizing Monoclonal Antibodies to the V3 Region of HIV-1 Can be Elicited by Peptide Immunization.** *Virology* 192:197.
14. Potts, B. J., K. G. Field, Y. Wu, M. Posner, L. Cavacini, and M. White-Scharf. 1993. **Synergistic Inhibition of HIV-1 by CD4 Binding Domain Reagents and V3-Directed Monoclonal Antibodies.** *Virology* 197:415.
15. M. Robert-Guroff, A. Louie, M. Myagkikh, F. Michaels, M. Paule Kieny, M. E. White-Scharf, B. Potts, D. Grogg, and M. S. Reitz, Jr. 1994. **Alteration of V3 Loop Context within the Envelope of Human Immunodeficiency Virus Type 1 Enhances Neutralization.** *Journal of Virology* 68:3459.

16. Kumagai-Braesch, M., B. Schacter, Z. Yan, J. Michaelson, S. Arn, M. Smith, M. White-Scharf, R. Monroy, D. H. Sachs, and J. T. Kurnick. 1995. **Identification of Swine and Primate Cellular Adhesion Molecules (CAM) Using Mouse Anti-Human Monoclonal Antibodies.** *Xenotransplantation* 2:88.

17. Latinne, D., B. De La Parra, Y. Nizet, A. Cornet, V. Giovino-Barry, R. L. Monroy, M. E. White-Scharf, and H. Bazin. 1996. **An Anti-CD2 mAb Induces Immunosuppression and Hyporesponsiveness of $CD2^+$ Human T Cells *In Vitro*.** *International Immunology* 8:1113.

18. Schad, V., J. L. Greenstein, V. Giovino-Barry, A. LeGuern, T. Matejic, R. Glaser, M. Dickerson, Y. Xu, H. Bazin, D. Latinne, R. Monroy, and M. E. White-Scharf. 1996. **An Anti-CD2 Monoclonal Antibody that Elicits Alloantigen-Specific Hyporesponsiveness.** *Transplantation Proceedings* 28:2051.

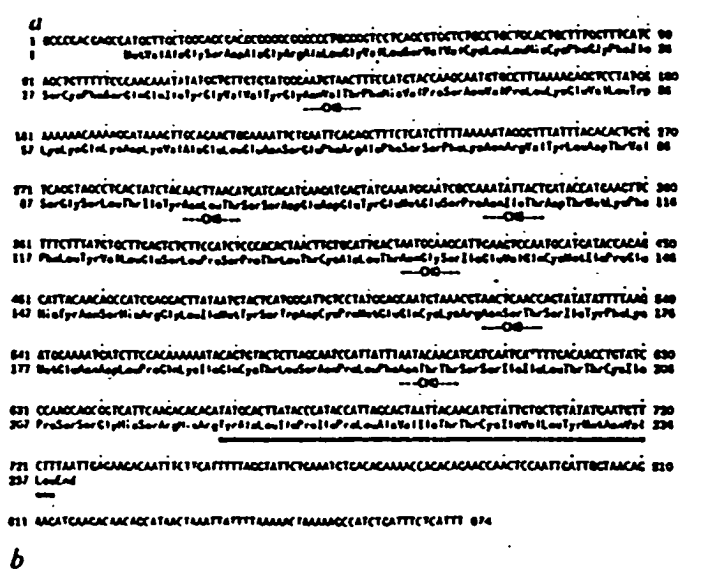

Fig. 3 *a*, Sequence of the LFA-3 cDNA. The sites of potential *N*-linked glycosylation are denoted by the symbol -CHO-; the hydrophobic carboxyl terminus is underscored. *b*, Hydropathicity profile of the amino-acid sequence in *a*.

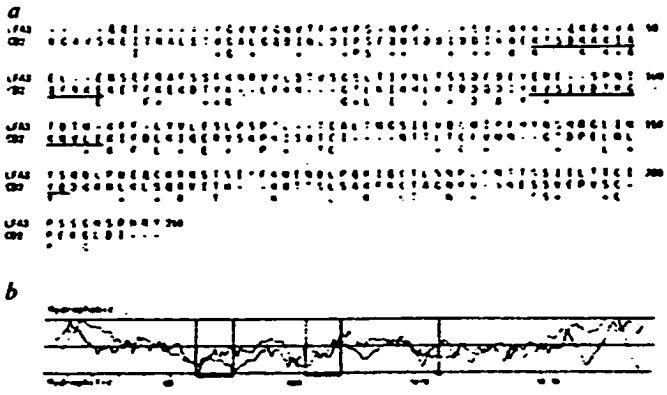

Fig. 4 *a*, Optimal alignment of the extracellular portions of LFA-3 and CD2 by the ALIGN program of the Protein Identification Resource (NBRF) (ref. 25). Conserved residues are displayed beneath the aligned sequences; asterisks were displayed if the residues were closely related. Epitope regions of CD2 identified elsewhere[28] are shown underlined. *b*, Superposition of the hydropathicity plots of LFA-3 and CD2. The amino-terminal sequences of both proteins were compared through the carboxy-terminal hydrophobic sequences. Solid bars denote the antigenic regions identified in CD2.

LFA-3 and CD2 using the ALIGN program of the NBRF sequence comparison package gave optimal alignment of the extracellular domains, as shown in Fig. 4. Monte Carlo simulation of the alignment of 500 randomly permuted variants of the two sequences gave a mean score 5.2 s.d. lower than the alignment score computed for LFA-3 and CD2, which corresponds to a probability of $\sim 10^{-7}$ for spontaneous occurrence of an equally good or better match between two proteins of identical composition[25]. As the homology extends throughout the external domain of the two molecules, the proteins could be distantly related. An alternative explanation, that convergent selective pressures have shaped essentially similar molecules from dissimilar archetypes, requires that multiple structural features of the two molecules be selected. Alignment of the hydropathicity profiles (Fig. 4) shows that, despite substantial divergence, the two proteins have strikingly similar gross organization. A precedent for the hypothetical ancestral progenitor may be found in the homotypic neural cell adhesion molecule NCAM, which adopts both phosphatidylinositol-linked and conventional transmembrane forms[26,27]. Moreover, CD2 is significantly homologous to two NCAM segments of ~200 residues which span domains II and III, and IV and V (ref. 7 and A. F. Williams, personal communication). Thus the heterotypic lymphoid and homotypic neural cell adhesion reactions could share a common evolutionary origin.

I thank Jen Sheen for assistance with the *in vitro* transcription and translation, Steven Herrmann for phospholipase C, Ivan Stamenkovic for the B-cell filter and B-cell and germ line DNA, Alan Williams for discussion and communication of the NCAM/CD2 homology, Steven Burakoff for TS2/9 antibody and encouragement, Thomas Hünig for the T11TS sequence, and David Simmons, Andrew Peterson, the referees, and members of the group for criticism. This work was supported by a grant from Hoechst AG.

Note added in proof: A cDNA encoding a transmembrane form of LFA-3 has recently been isolated (B. Wallner *et al. J. exp. Med.*, in the press).

Received 8 June; accepted 22 September 1987.

1. Plunkett, M. L., Sanders, M. E., Selvaraj, P., Dustin, M. L. & Springer, T. A. *J. exp. Med.* 165, 664–676 (1987).
2. Selvaraj, P. *et al. Nature* 326, 400–403 (1987).
3. Krensky, A. M., Robbins, E., Springer, T. A. & Burakoff, S. *J. J. Immun.* 132, 2180–2182 (1984).
4. Shaw, S. *et al. Nature* 323, 262–264 (1986).
5. Vollger, L. W., Tuck, D. T., Springer, T. A., Haynes, B. F. & Singer, K. H. *J. Immun.* 138, 358–363 (1987).
6. Hünig, T. R., Tiefenthaler, G., Meyer zum Büschenfelde, K.-H. & Meuer, S. C. *Nature* 326, 298–301 (1987).
7. Williams, A. F. *Immun. Today* 8, 298–303 (1987).
8. Sanchez-Madrid, F. *et al. Proc. natn. Acad. Sci. U.S.A.* 79, 7489–7493 (1982).
9. Krensky, A. M. *et al. J. Immun.* 131, 611–616 (1983).
10. Dustin, M. L., Sanders, M. E., Shaw, S. & Springer, T. A. *J. exp. Med.* 165, 677–692 (1987).
11. Seed, B. & Aruffo, A. *Proc. natn. Acad. Sci. U.S.A.* 84, 3365–3369 (1987).
12. van Dongen, J. J. M. *et al. J. Immun.* 135, 3144–3150 (1985).
13. Hünig, T. *J. exp. Med.* 162, 890–901 (1985).
14. Aruffo, A. & Seed, B. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
15. Dailey, L. & Basilico, C. *J. Virol.* 54, 739–749 (1985).
16. Barbosa, J. A. *et al. J. Immun.* 136, 3085–3091 (1986).
17. Kozak, M. *Microbiol. Rev.* 47, 1–45 (1983).
18. von Heijne, G. *Nucleic Acids Res.* 14, 4683–4690 (1986).
19. Low, M. G. & Kincade, P. A. *Nature* 318, 62–64 (1985).
20. Tse, A. G. D., Barclay, A. N., Watts, A. & Williams, A. F. *Science* 230, 1003–1008 (1985).
21. Low, M. G., Ferguson, M. A. J., Futerman, A. H. & Silman, I. *Trends biochem. Sci.* 11, 212–215 (1986).
22. Berger, J., Howard, A. D., Gerber, L., Callen, B. R. & Udenfried, S. *Proc. natn. Acad. Sci. U.S.A.* 84, 4825–4829 (1987).
23. Sewell, W. A., Brown, M. H., Dunne, J., Owen, M. J. & Crumpton, M. J. *Proc. natn. Acad. Sci. U.S.A.* 83, 8718–8722 (1986).
24. Sayre, P. H. *et al. Proc. natn. Acad. Sci. U.S.A.* 84, 2941–2945 (1987).
25. Dayhoff, M. O., Barker, W. C. & Hunt, L. T. *Meth. Enzym.* 91, 524–545 (1983).
26. He, H. T., Barbet, J., Chaix, J. C. & Goridis, C. *EMBO J.* 5, 2489–2494 (1986).
27. Cunningham, B. A., Hemperly, J. J., Murray, B. A., Prediger, E. A., Brackenbury, R. & Edelman, G. M. *Science* 236, 799–806 (1987).
28. Peterson, A. & Seed, B. *Nature* 329, 842–846 (1982).

Monoclonal antibody and ligand binding sites of the T cell erythrocyte receptor (CD2)

Andrew Peterson & Brian Seed

Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA

The human T cell erythrocyte receptor (CD2 antigen) allows thymocytes and mature T cells to adhere to thymic epithelium and target cells through a cell surface protein, LFA-3 (refs 1–3). Monoclonal antibodies recognizing CD2 can either block adhesion or, in certain combinations, induce an antigen-independent T cell activation[7–9]. We have identified the binding sites for 16 monoclonal antibodies against CD2 by a rapid and generally applicable

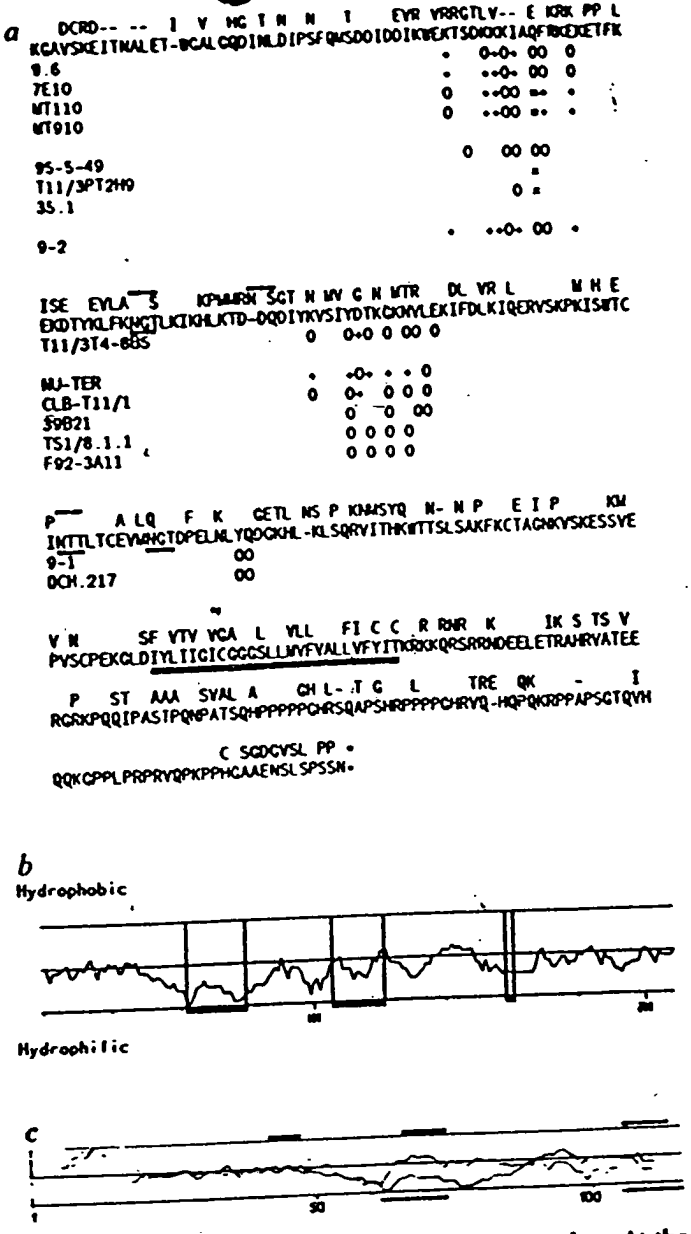

Fig. 1 *a*, The primary amino-acid sequence of the human CD2 protein[4,24,25]. The mouse sequence is shown above the human sequence only where it differs from the human sequence[26]. The large bar indicates the extent of the transmembrane domain. The smaller bars above the amino-acid sequence indicate potential *N*-linked glycosylation sites in the mouse sequence; those below indicate potential sites in the human sequence. The antibodies used are shown along the left margin. The 0 symbol under the primary sequence indicates either that a mutant has a substitution at that position or that indirect immunofluorescence of a mutant obtained with another antibody showed loss of reactivity; + indicates retention of reactivity for all variants examined and = indicates that only a proline substitution at that position affects reactivity *b*, Hydropathicity profile of the first 190 amino acids of CD2 (the extracellular domain) showing the location of the three epitopic regions. *c*, Superposition of the hydropathicity profiles of the first 115 residues of CD2 and the human immunoglobulin κ variable region, Vh (V-III) (ref. 31). κ V-region hypervariable sequences are shown as black bars above the profile, and CD2 ligand binding domains as black bars below the profile. Alignment of the domains shown gives an ALIGN score[32] of 3.7 s.d. above the mean, corresponding to a probability of $\approx 10^{-4}$ for spontaneous occurrence of an equally good or better match.

Methods. The 600 nucleotides of CD2 sequence following position 63 of ref. 6 were synthesized in a collection of twenty 33-mer oligonucleotides, each overlapping its predecessor by three bases. The monomer reagents for the synthesis contained 95% of the wild-type base and 5% of a mixtures of the other 3 bases at each position. A pool of mutants was obtained from each mutagenized 33-mer by oligonucleotide directed mutagenesis of #H3MCD2 (ref. 27) as described[28,29] except that AMV reverse transcriptase was used instead of T4 DNA polymerase. Mutants were selected following spheroplast fusion into COS cells. 48 h post-fusion the COS cells were removed from the culture dish using PBS containing 5 mM EDTA. Antibody incubations and washes were performed as described[6]. The cells were incubated with 0.1% by volume of the negative selection antibody, washed, incubated with 5 μg ml^{-1} of rabbit anti-mouse immunoglobulin antibody (Rockland), washed and incubated for 30 min at 37 °C in 2 mls of 50% rabbit complement (Pel-Freez), 50% Dulbecco's media (GIBCO). After complement lysis the cells were washed, incubated with the positive selection antibody, washed and added to goat anti-mouse immunoglobulin coated dishes as described[6]. Cells adhering to the dish were lysed and the recovered plasmid DNA was transformed into *E. coli*. Mutants were identified by DEAE dextran transfection of COS cells in a 35 mm well with 20% of the plasmid DNA from a 1.5 ml mimiprep. The cells were assayed sequentially for binding of the negative and positive selection antibody 48 h post transfection by indirect immunofluorescence. Mutants were sequenced using the chain termination method[30]. In all cases the mutations fell within the span of a single oligonucleotide.

mutational analysis. The binding sites fall in three discrete regions: antibodies that participate in activation and block erythrocyte adhesion bind to the first region; antibodies that block adhesion bind to the second region; and antibodies that participate in activation but do not block adhesion bind to the third region. A large number of mutations selected for loss of antibody reactivity in the first two regions also weaken the CD2–LFA-3 interaction. Good agreement was observed between mutational lesions blocking LFA-3 binding and lesions blocking binding by activating antibodies, which supports the view that such antibodies induce T cell activation by mimicking the effect of LFA-3 binding. CD2 sequences that participate in LFA-3 binding correspond to immunoglobulin variable region hypervariable sequences when the homologous domains are aligned.

To isolate epitope loss mutants, COS cells were transfected with a pool of mutagenized plasmids, cultured for 48 hours, collected and sequentially treated with an anti-CD2 monoclonal antibody, rabbit anti-mouse immunoglobulin antibody, and complement. Because spontaneous deletion mutants arise frequently in COS cells[10,11], a positive selection step was included; the cells spared by complement treatment were treated with antibody recognizing a distinct CD2 epitope(s) and allowed to adhere to dishes coated with goat anti-mouse immunoglobulin antibody[12]. Plasmid DNA recovered from the adherent cells[13] was transformed into *Escherichia coli*, amplified, and reintroduced into COS cells for further rounds as appropriate. At the end of the selection process DNA from individual bacterial colonies was transfected into COS cells which were then scored for antibody binding. The antibodies used for mutant isolation are shown in Table 1. The results of the mutant selections are summarized in Figs 1 and 2.

The mutants are described below by a wild-type residue/mutant residue convention, so that Lys-48Asn, for example, means that the lysine at position 48 has been replaced with an asparagine. 114 Primary mutants were isolated, resulting in a collection of 47 different amino-acid sequence variants. The variation falls in three discrete regions. Region 1 is centred about Lys48 and contains mutations for the antibodies (9.6, 7E10, MT110 and MT910; group I antibodies) which, together with mAb 9-1, can induce IL-2 synthesis in T cells (B. Bierer and A.P., unpublished observations and ref. 14). All but one (9-2) of the other antibodies giving mutations in region 1 have been reported to induce IL-2 receptors but not IL-2 in collaboration with mAb 9-1 (ref. 14). Region 2 is centred about Gly95. Most of the antibodies recognizing region 2 have little effect on T cell activation when used with mAb 9-1. Region 3 is represented by a single mutation which causes loss of reactivity with both 9-1 and OKT217.

The ability of the mutant CD2 proteins to promote adhesion of human erythrocytes to transfected COS cells mediated by

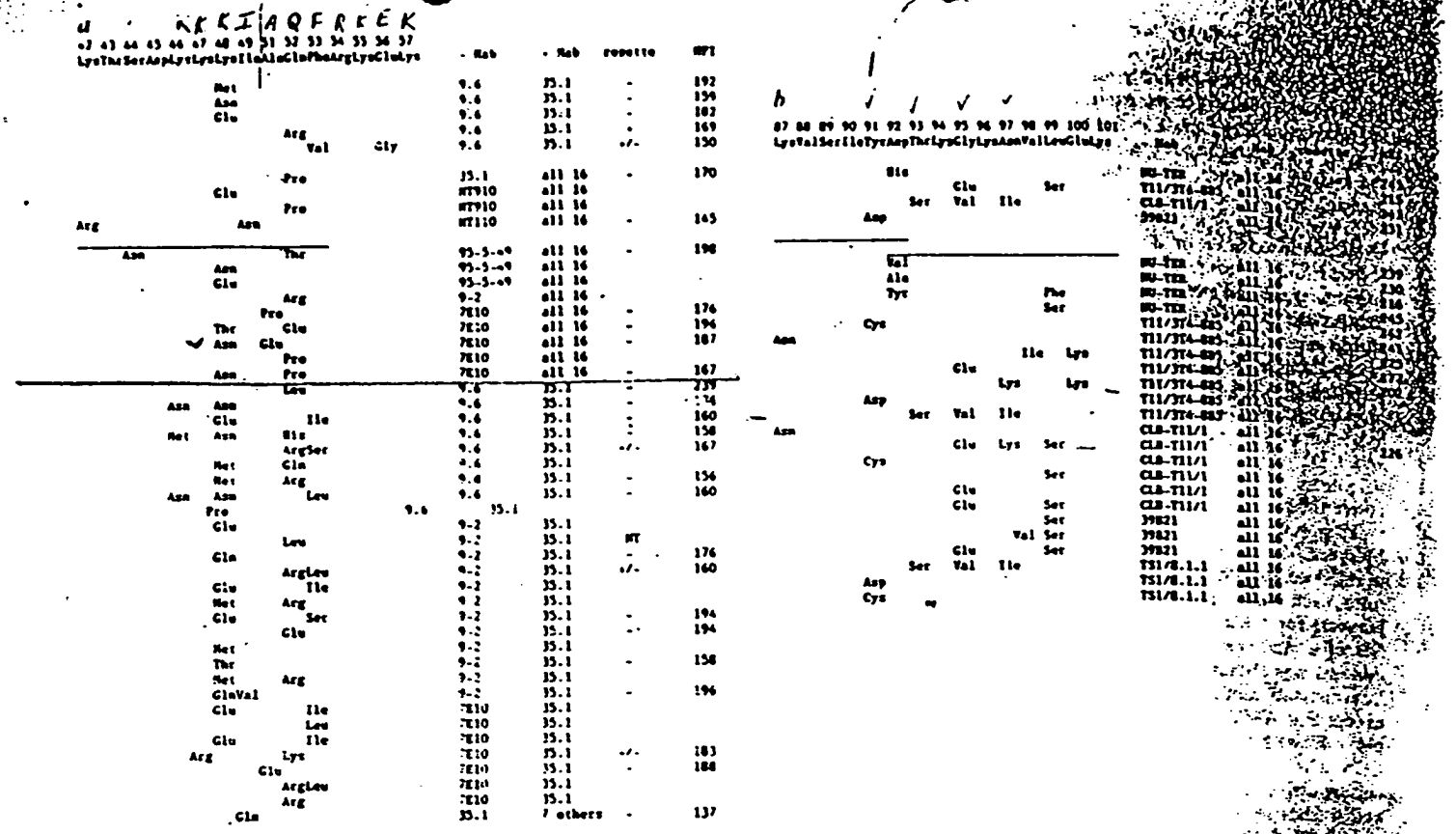

Fig. 2 *a*, The mutant collection defining epitope region 1. CD2 residues 42–57 are shown above the amino-acid substitution encoded by each mutant. The first column on the right shows the antibody used for negative selection, the second column shows the positive selection antibody(s). 'All 16' indicates that all 16 monoclonals in Table 1 were combined and used for the positive-selection step. '7 others' indicates that the 7 antibodies recognizing region 1 were combined and used for the positive-selection step. The third column shows the erythrocyte rosetting phenotype of the mutant: + wild type, +/− partial rosetting, − no detectable rosetting (see Fig. 3). A blank denotes a variant shown elsewhere in the table. *These mutants rosetted much more poorly than the others scored as +/−. The column on the far right shows the mean fluorescence intensity MFI measured by flow cytometry of COS cells expressing the mutant CD2s. *b*, The mutant collection defining epitope region 2. CD2 residues 86–101 are shown above the mutant substitutions. Other notations are as in *a*.
Methods. Cells stained by indirect immunofluorescence were judged to express antigen if ≥3% of the transfected population had an MFI ≥40. Only 0.1% of mock-transfected cells had an MFI ≥ 40, and the mean MFI of the remainder was 4. COS cells transfected with wild-type CD2 gave MFI values of 179 for mAb Nu-Ter (used for region 1 mutants) and 239 for mAb 35.1 (used for region 2 mutants). The mutants listed directly under the primary sequence were from a pool of plasmids mutagenized by oligonucleotides spanning the extracellular domain.
The mutants listed under the bar were obtained using plasmids mutagenized by oligonucleotides encoding the span of the bar.

LFA-3 was measured by a qualitative erthyrocyte rosette assay. Three phenotypes were scored: wild-type, partial, and non-rosetting, as illustrated in Fig. 3 and summarized in Fig. 2. Many of the mutations leading to changes in regions 1 and 2 dramatically reduced rosetting. To examine this further, a few mutants were created by specific oligonucleotide mutagenesis. Substitution of asparagine or alanine for lysine at each of positions 46, 47, and 48 demonstrated a striking correlation between the binding of antibody mAb 9.6 and erythrocyte adhesion; Lys46Asn/Ala showed a modest effect on both Mab 9.6 and erthrocyte binding, Lys47Asn/Ala had no effect on either, and Lys48Asn/Ala completely abolished both (Fig. 3*b*). Similarly, residue 51 was important for both erthrocyte and 9.6 binding, whereas residue 52 had only a weak effect on each.

Using different antibodies and mutants, we have also shown that Lys48 is important in the interaction of CD2 with group I antibodies and with LFA-3 (Figs 1, 2 and 3). For example, the mutant Lys48Glu is unreactive with all the group I antibodies, and none of the molecules substituted at Lys48 has any detectable rosetting activity. The behaviour of molecules with substitutions at Lys48 supports the idea that group I antibodies mimic the effect of LFA-3 binding in provoking T cell proliferation.

Although some residues can directly determine antibody reactivity, others of secondary importance for antibody [illegible] can be identified, because they are frequently altered in [illegible] with other changes. For example, a Lys46Asn substitution is frequently found in mutants which do not bind [illegible] but by itself it has little effect on antibody binding. [illegible] phenomenon may be present in the repeated isolation [illegible] 51, 52 double mutants (Fig. 2).

Amino-acid substitutions could lead to loss of [illegible] LFA-3 binding either by elimination of a specific [illegible] by causing a local denaturation. Some patterns of [illegible] binding or rosetting argue against this latter possibility. For example, all the molecules substituted at Lys48 still bind [illegible] 35.1, which is sensitive to changes at Ile49. Similarly, [illegible] mAb 9.6 or LFA-3 can bind the Gln51Leu variant [illegible] recognized by antibodies 7E10 and 9-2. A Gln51Arg [illegible] unreactive with 7E10 and 9-2, but rosettes erthrocytes [illegible] same way as the wild type. In the second epitope [illegible] Tyr91Asp causes loss of rosetting, but antibody Nu-Ter [illegible] is unaffected, even though many substitutions at [illegible] eliminate Nu-Ter reactivity.

However, a Gln51Pro substitution may induce a local [illegible] ation, since proline residues restrict alpha helix formation [illegible] none of the antibodies recognizing the first region [illegible]

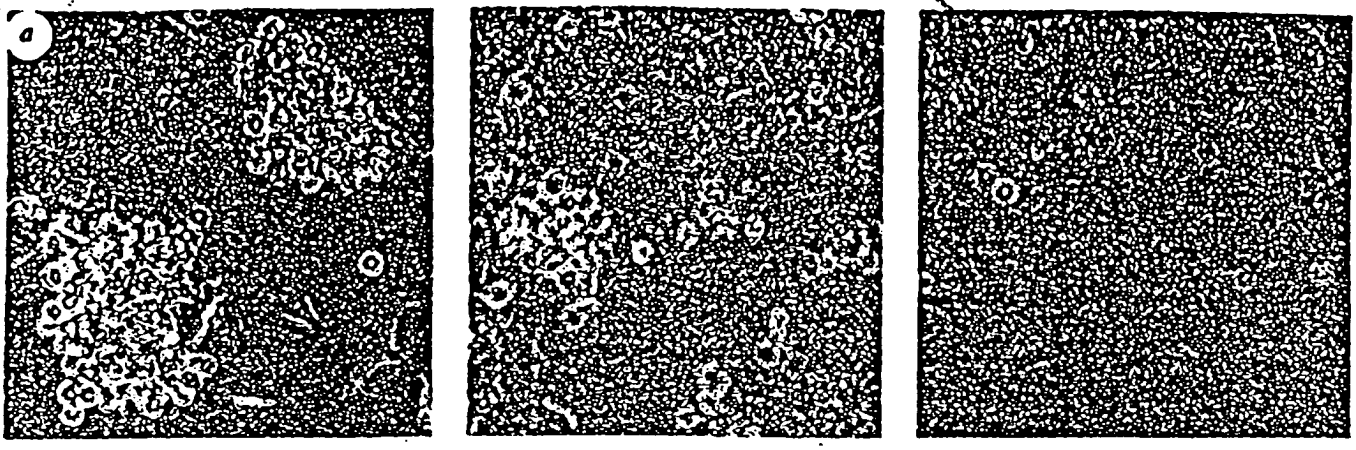

Fig. 3 *a*, Adhesion of human erthrocytes to transfected COS cells. The left photograph shows wild-type rosettes, the centre shows partial rosettes and the right photograph no rosettes. Wild-type rosettes completely obscure the transfected COS cells, and are macroscopically visible. Partial rosettes are microscopically visible and leave some transfected COS cells exposed. Absence of rosetting was scored if the mutant was indistinguisable from a negative control (CD8 expressing COS cells), that is, no rosettes were found after careful scanning of the plate. *b*, sensitivity of erythrocyte rosetting and 9.6 binding to changes at specific amino-acid positions. + indicates that rosetting or binding is retained upon substitution at that position. +/− indicates that substitution has some effect, namely a partial rosetting phenotype or minimal effect on antibody binding. − indicates that rosetting or antibody binding is eliminated by a single amino-acid substitution at that position.

Gln51Pro. mAbs 35.1 and T11/3PT2H9 gave Gln51Pro exclusively when all 16 antibodies were used for positive selection. Because frequent isolation of Gln51Pro was observed with other mAbs, many of the mutants in the first epitopic region (Figs 1 and 2) were isolated using mAb 35.1 as the only positive selection antibody.

To isolate a 35.1$^-$ mutant other than Gln51Pro, only the antibodies failing to bind to this variant were used for positive selection. Three cycles of enrichment gave a single 35.1$^-$ Ile49Gln mutant altered in all three bases of the original codon. This unusual mutation suggests that the affinity of 35.1 antibody derives from multiple conformational features of CD2, so that substitution for a single feature only rarely greatly decreases affinity. The Gln51Pro mutation may eliminate several of these interactions by gross alteration of the local secondary structure. Because the affinity of the 35.1 antibody is comparable to that of antibody 9.6 (ref. 16), the unusual mutational pattern of this antibody probably arises from a different type of binding and not simply from a stronger interaction.

Only one mutant was found with the two antibodies recognizing region 3, a Tyr140Asn and Gln141His double substitution. Both of these antibodies, however, react only weakly with the CD2 molecule expressed on COS cells, which compares with their weak reactivity with CD2 on unactivated T cells[14]. Previous activation of T cells or incubation with a group I antibody is necessary to make the 9-1 epitope available[14]. The rapid acquisition of mAb 9-1 reactivity suggests that it is caused by a conformational change in the molecule and not by *de novo* synthesis of a different species[17].

To further study the interaction of antibodies with each of the two major antibody-binding regions, a large number of mutants were isolated using a CD2 preparation mutagenized by only one or a few oligonucleotides (Fig. 2; see also Fig. 1 legend). Mutants were obtained from such plasmid pools at a frequency of 75–100% after a single round of selection. This allowed a large number of amino-acid variants to be quickly isolated. In the first epitope region the antibodies 7E10, 9-2 and 9.6 were chosen for intensive study because they appear to contact many of the same amino-acid residues (Figs 1 and 2). Two of the antibodies can function, together with mAb 9-1, in T cell activation but the third (9-2) cannot[14]. Each antibody gave rise to a slightly different range of mutations (Fig. 2): the 9-2$^-$ mutations span only 5 residues compared to 8 for the 7E10$^-$ mutations and 10 for the 9.6$^-$ mutations. 9-2 is the only IgM antibody which recognizes region 1, and its inability to activate could be due to a decreased affinity, or to steric interference with 9-1. A large number of mutants were similarly isolated in the second epitopic region (Fig. 2).

Table 1 Antibodies

Antibody	Isotype
9.6	IgG_{2a}
7E10	IgG_{2b}
MT910	IgG_1
MT110	IgG_1
95-5-49	?
35.1	IgG_{2a}
T11/3PT2H9	IgG_1
T11/3T4-8B5	IgG_{2a}
9-2	IgM
Nu-Ter	IgG_1
CLB-T11/1	IgG_1
39B21	IgG^*_{2a}
TS1/8.1.1	IgG_1
F92-3A11	IgG_1
9-1	IgG_3
OCH217	IgM

A partial panel of anti-CD2 monoclonal antibodies was obtained. The first four antibodies (9.6, 7E10, MT910, MT110) can each induce IL-2 release from T cells expressing CD2 in the presence of antibody 9-1. A more complete functional analysis of the antibodies can be found in ref. 14.

* Antibody 39B21 is a rat monoclonal and all others are mouse antibodies.

It has been proposed that [illegible] mediates both cell-cell adhesion and antigen-independent activation reactions. The former function is well-established[2,3,18], but the case for the latter still rests on the unique properties of antibodies such as 9-1 in triggering proliferation in the presence of either group I antibodies[7,14] or sheep erthrocytes[19-21]. The first epitope region we have identified is probably important in both the adhesion and activation functions of CD2. Binding of the first region by antibodies allows CD2 to respond to subsequent binding of antibody 9-1 and we predict that LFA-3 binding to CD2 would allow comparable response to 9-1. If so, the adhesion and activation mediated by CD2 are intimately related and not distinct functions.

Because 9-1 does not block erythrocyte rosetting, and because a CD2 variant which does not react with mAb 9-1 still binds erythrocytes, it is unlikely that LFA-3 binding alone can cause activation; further analysis of the region recognized by 9-1 antibodies is necessary for insight into the activation mechanism.

Recently the case for inclusion of CD2 in the immunoglobulin superfamily[22] has been strengthened by discovery of highly significant homologies between CD2 and non-immunoglobulin members of the family[23]. Alignment of the N-terminal 115 residues of CD2 with immunoglobulin κ variable sequences shows that the CD2 regions 1 and 2 correspond to the locations of light chain hypervariable (antibody-combining site) regions 2 and 3 (Fig. 1). This suggests that CD2 ligand-binding sites are phylogenetically related to variable region-combining sites, and supports the idea that adhesion interactions between members of the immunoglobulin superfamily can mimic antibody-antigen interaction.

We thank Barbara Bierer, Steve Burakoff and Sandro Aruffo for discussion, Mary Honma and Dave Simmons for comments on the manuscript, Bill Sewell and Mike Crumpton for a preprint of their work, Neil Clipstone for a sequence reading, Lerzan Kizilay and John Smith for oligonucleotides and Ivan Stamenkovic for discussion and blood sampling. We thank the organizers and participants of the Third International Workshop on Human Leukocyte Differentiation Antigens for making antibodies available. This work was supported by a grant from Hoechst AG.

Received 6 June; accepted 22 September 1987.

1. Vollger, L. W., Tuck, D. T., Springer, T. A., Haynes, B. F. & Singer, K. H. *J. Immun.* **138**, 358–363 (1987).
2. Shaw, S. *et al. Nature* **323**, 262–264 (1986).
3. Krensky, A. M., Robbins, E., Springer, T. A. & Burakoff, S. J. *J. Immun.* **132**, 2180–2182 (1984).
4. Dustin, M. L., Sanders, M. E., Shaw, S. & Springer, T. A. *J. exp. Med.* **165**, 677–692 (1987).
5. Selvaraj, P. *et al. Nature* **326**, 400–403 (1987).
6. Seed, B. & Aruffo, A. *Proc. natn. Acad. Sci. U.S.A.* **84**, 3365–3369 (1987).
7. Meuer, S. C. *et al. Cell* **36**, 897–906 (1984).
8. Olive, D. *et al. Eur. J. Immun.* **16**, 1063–1068 (1986).
9. Huet, S. *et al. J. Immun.* **137**, 1420–1424 (1986).
10. Calos, M. P., Lebkowski, J. S. & Botchan, M. R. *Proc. natn. Sci. U.S.A.* **80**, 3015–3019 (1983).
11. Razzaque, A., Mizuguwa, H. & Seidman, M. M. *Proc. natn. Sci. U.S.A.* **80**, 3010–3014 (1983).
12. Wysocki, L. J. & Sato, V. L. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2844–2848 (1978).
13. Hirt, B. *J. molec. Biol.* **26**, 365–369 (1967).
14. Yang, S. Y. Rhee, S., Angelos, G. & DuPoint, B in *Leukocyte Typing. III* (ed. McMichael, A. J.) 113–116 (Oxford University Press, 1987).
15. Chou, P. Y. & Fasman, G. D. *A. Rev. Biochem.* **47**, 251–276 (1978).
16. Martin, P. J. *et al. J. Immun.* **134**, 180–185 (1985).
17. Yang, S. Y., Chuntz, S. & Dupoint, B. *J. Immun.* **137**, 1097–1100 (1986).
18. Sanchez-Madrid, F. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7489–7493 (1982).
19. Hünig, T. R., Tiefenthaler, G., Meyer zum Büschenfelde, K.-H. & Meuer, S. C. *Nature* **326**, 298–301 (1987).
20. Hünig, T. *J. exp. Med.* **162**, 890–901 (1985).
21. Hünig, T. R. *J. Immun.* **136**, 2103–2108 (1986).
22. Williams, A. F., Barclay, A. N., Clark, S. J., Paterson, D. J. & Willis, A. C. *J. exp. Med.* **165**, 368–380 (1987).
23. Williams, A. F. *Immun. Today* **8**, 298–303 (1987).
24. Sewell, W. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **83**, 8718–8722 (1986).
25. Sayre, P. H. *et al. Proc. natn. Acad. Sci. U.S.A.* **84**, 2941–2945 (1987).
26. Sewell, W. A. *et al. Eur. J. Immun.* **17**, 1015–1020 (1987).
27. Aruffo, A. & Seed, B. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
28. Kunkel, T. A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 488–492 (1985).
29. Levinson, A., Silver, D. & Seed, B. *J. molec. appl. Genet.* **2**, 507–517 (1984).
30. Sanger, F., Nicklen, S. & Coulson, A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
31. Pech, M. & Zachau, H. G. *Nucleic Acids Res.* **12**, 9229–9236 (1984).
32. Dayhoff, M. O., Barker, W. C. & Hunt, L. T. *Meth. Enzym.* **91**, 524–545 (1983).

Anchoring mechanisms for LFA-3 cell adhesion glycoprotein at membrane surface

Michael L. Dustin, Periasamy Selvaraj, Robert J. Mattaliano* & Timothy A. Springer

Laboratory of Membrane Immunochemistry, Dana Farber Cancer Institute, and Harvard Medical School, Boston Massachusetts 02115, and * Biogen Research Corp, Cambridge, Massachusetts 02142, USA

The manner in which a membrane protein is anchored to the lipid bilayer may have a profound influence on its function. Most cell surface membrane proteins are anchored by a membrane-spanning segment(s) of the polypeptide chain, but another type of anchor has been described for several proteins: a phosphatidyl inositol glycan moiety, attached to the protein C terminus[1,2]. This type of linkage has been identified on membrane proteins involved in adhesion[3] and transmembrane signalling[4,5] and could be important in the execution of these functions. We report here that an immunologically important adhesion glycoprotein, lymphocyte function-associated antigen 3 (LFA-3), can be anchored to the membrane by both types of mechanism. These two distinct cell surface forms of LFA-3 are derived from different biosynthetic precursors. The existence of a phosphatidyl-inositol-linked and a transmembrane anchored form of LFA-3 has important implications for adhesion and transmembrane signalling by LFA-3.

LFA-3 is a cell-surface glycoprotein found on erythrocytes, epithelial cells, endothelial cells, fibroblasts and most cells of haematopoietic origin[6]. LFA-3 interacts with the T lymphocyte CD2 membrane glycoprotein, and this ligand-receptor pair mediates intercellular adhesion between LFA-3$^+$ cells and thymocytes, natural killer cells, cytolytic T lymphocytes, and other mature T lymphocytes[6–11]. Cell surface LFA-3 and LFA-3 reconstituted into artificial membranes can both activate T lymphocytes in conjunction with other signals[12,13], which is consistent with the ability of pairwise combinations of anti-LFA-3 monoclonal antibody (MAb) to activate CD2$^+$ cells[6]. Recently we have found that LFA-3 is deficient in affected erythrocytes in patients with paroxysmal nocturnal haemoglobinuria (PNH) (ref. 14), an acquired disorder affecting phosphatidyl inositol (PI)-linked proteins[15]. This suggests that LFA-3 is linked to the surface of human erythrocytes by a PI-glycan anchor.

We first obtained evidence for distinct forms of LFA-3 by studying its biosynthesis in the JY B lymphoblastoid cell line. Labelling of JY cells for one minute with [illegible] followed by a five minute chase and isolation with anti-LFA-3 Sepharose, revealed two distinct LFA-3 precursors of apparent molecular mass (M_r) of 41,000 (41K) and 37,000 (37K) (Fig. 1a, lane 2 arrows). Chase for 10 and 20 minutes showed a decrease in size of the precursors to 39K and 35K (Fig. 1a, lanes 3 and 4), which is probably due to trimming of high-mannose oligosaccharides from high-mannose oligosaccharides. There was no apparent interconversion of the two precursors during biosynthesis at 24 °C. After chase for 20 h, the precursors were converted to the mature form of LFA-3, which migrated as a broad band of mean size 65K (Fig. 1a, lanes 4 and 5) and corresponded to the form surface-labelled with ^{125}I (ref. 6).

Endoglycosidase H (Endo H) treatment of the [illegible] LFA-3 precursors resulted in two bands of [illegible] intensity of 29K (p29) and 25.5K (p25.5) respectively (Fig. 1b, lanes 1 and 2), but had no effect on mature LFA-3 (Fig. 1b, lanes 5 and 6). Therefore each LFA-3 precursor contains a number of high mannose *N*-linked oligosaccharides which are converted to endo H-resistant complex *N*-linked oligosaccharides during glycoprotein maturation. *N*-glycanase treatment [illegible] the precursor and mature forms of LFA-3 to the [illegible]